The South African Journal
of
Medical Laboratory Technology

ORGAN OF THE SOCIETY OF MEDICAL LABORATORY
TECHNOLOGISTS OF SOUTH AFRICA

Vol. 3, No. 4 A QUARTERLY September, 1957

EDITOR:
CECIL R. STUART

Single Numbers 5/6 Annual Subscription £1/1/-

SOCIETY OF MEDICAL LABORATORY TECHNOLOGISTS OF SOUTH AFRICA

—o—

President: Professor E. H. CLUVER

—o—

COUNCIL

Chairman: Dr. F. A. BRANDT

Mr. J. MAYTHAM — Mr. A. SCOTT

Mr. G. TURNER — Mr. P. ROUX

Mr. P. N. BUCK — Mr. G. C. BUCKLE

General Secretary-Treasurer: Mr. G. W. WIKELEY

—o—

BRANCH SECRETARIES

Natal: Mr. P. N. BUCK — **Cape:** Mr. J. MAYTHAM

Southern Transvaal: Mrs. G. TOMLIN

—o—

Editor: CECIL R. STUART

Editorial Committee

Mr. W. G. POWELL — Mr. R. HORNER

Mr. J. HERRICK — Miss I. E. MULLER

Mr. A. J. S. GREENFIELD

Printed by Provincial Printing Co. (Pyt.), Ltd., 482 Smith Street, Durban, and published by the Society of Medical Laboratory Technologists of South Africa, c/o Central Pathological Laboratory, Private Bag, Jacobs, Natal.

JENA GLASS

a trade mark of distinction,
member of the ZEISS group
of firms

We have pleasure
in announcing
that we have now established a

Special Department

under the management of a factory expert. Whatever your "glass-problems" might be, discuss them with our Mr. K. Harth, late of Schott & Gen., who is entirely at your disposal.

ZEISS from the PUNKTAL spectacle lens to the ZEISS Planetarium

OPTICAL INSTRUMENTS (PTY.) LTD., Johannesburg
Maritime House, 97a Main Street, Box 1561, Telephone 34-3980/88/89
ZEISS Representatives for S.A. and the Central African Federation

ARE YOU INTERESTED IN ANIMALS WITHOUT BEING A FANATIC?

Then write for the illustrated prospectus of

UFAW

(The Universities Federation for Animal Welfare)

7a Lamb's Conduit Passage, London, W.C.1

UFAW fosters consideration for the physical and mental comfort of experimental animals, the avoidance of procedures which involve serious suffering, and the development of techniques calculated to reduce discomfort to a minimum.

Publications include:—

The UFAW Handbook on the Care and Management of Laboratory Animals, edited by A. N. Worden, M.A., B.Sc., M.R.C.V.S., F.R.I.C (Ballière, Tindall & Cox. 31s. 6d.).

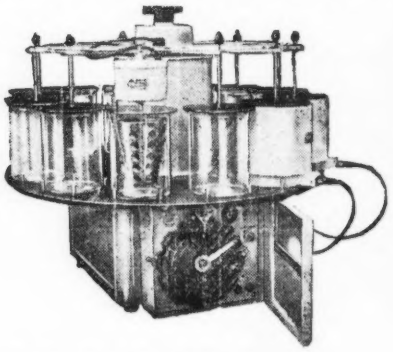
Kind Killing, compiled by F. Jean Vinter, M.D., Practical instruction in euthanasia for animals. Enlarged 3rd edition, 6d.

How to Befriend Laboratory Animals, by C. W. Hume, M.C., B.Sc., 3d.

Suggestions for the Legal Protection of Laboratory Animals Overseas, gratis.

ANNUAL MEMBERSHIP FEE: **Technicians 5/-; Student Technicians 1/-**

M.S.E. HISTOKINETTE



S. A. Agents and Stockists:

MACDONALD ADAMS & Co.

JOHANNESBURG
P.O. Box 68 - Phone 238161

DURBAN
P.O. Box 1807 - Phone 60888

Stability!

AMIDST THE SEA OF RISING COSTS CONSIDER **GURR'S** RECORD IN MICROSCOPICAL STAIN PRICES.

	1919 list	Current list
Methylene blue 10 g.	2/9	**2/6**
Leishman stain 5 g.	8/-	**3/-**

TWO EXAMPLES FROM MANY

Growing demand has enabled us generally to whittle prices—and you have shared the benefit of nearly 40 years.

Please forgive us this dissertation on our own efficiency—Your confidence and support have been vital, have always been appreciated, and will enable us to continue to bring you the best. Our slogan in 1919—"Stipulate Gurr's"—is still the best advice.

Ask for Catalogue E 56

GEORGE T. GURR LTD.

136/138 NEW KINGS ROAD - LONDON, S.W.6

Sole Agents for the Union of South Africa . . . . B. Owen Jones Limited

JOHANNESBURG P.O. Box 9955 | CAPE TOWN P.O. Box 434 | DURBAN P.O. Box 557

STUART JONES & DAVID ANDERSON LTD.

20 QUEEN STREET - DURBAN

•

WE ARE PROUD OF THE FACT THAT WE HAVE SUPPLIED MUCH OF THE EQUIPMENT NOW IN USE IN THE LABORATORIES OF THE NATAL PROVINCIAL ADMINISTRATION

We extend a cordial invitation to visit our showroom at the above address

Natal Agents for-

Baird & Tatlock (London) Limited, W. Edwards & Co. (London) Limited, Hilger & Watts Limited, Hopkin & Williams Limited, Watson & Sons Limited, George T. Gurr. Limited

COOKE, TROUGHTON & SIMMS

have pleasure

in informing our many friends

that we now operate in South Africa

as

Cooke, Troughton & Simms South Africa (Pty.) Ltd.

★

With our comprehensive range of microscopes we now offer apparatus for—

Blood cell and particle counting

Tissue processing

High and low power micromanipulations.

Full details on application

★

The South African Journal
of
Medical Laboratory Technology

ORGAN OF THE SOCIETY OF MEDICAL LABORATORY
TECHNOLOGISTS OF SOUTH AFRICA

Vol 3, No. 4 A QUARTERLY September, 1957

CONTENTS

	Page
Editorial	2
Redaksioneel	3
Obituary	4
The Coombes Test and Its Application in Pathology	5
Megaloblastic Anaemias	35
The Teaching of Biochemistry to Apprentices	37
Readers' Forum	39
Vacancies	41
Abstract	43
Notice	43

SUPPORT THE FIRMS WHO SUPPORT YOUR SOCIETY'S JOURNAL

Editorial

STATUS, QUALIFICATION, RESPONSIBILITY

Medical laboratory technologists today are cinderellas in the scientific world; particularly is this so in the auxiliary medical field where very often the old-fashioned "bottle washing mentality" is still found. The confusion which exists between the functions of the medical laboratory technologist and the laboratory steward or laboratory attendant in some quarters is one which is very difficult to understand in these enlightened days.

The medical laboratory technologist of today has undergone a long and arduous training and has become familiar with the multiplicity of techniques which are employed in the medical laboratory. His training has been over a period of five years, and during this time he has passed professional examinations of an extremely high standard—sufficiently so for some countries to recognise this as graduate level education.

How then is the medical technologist inferior to the science graduate who, with a major in some odd science, enters medical laboratory technology? Why then is the unexperienced graduate often preferred to the highly trained technologist ? Why then are there no facilities for further study in the subjects covered by modern medical technology ?

The modern medical laboratory technologist is capable of organising and controlling a clinical laboratory alone, and often does so. His work is relied upon by the clinical staff and he is regarded as a responsible member of the hospital staff.

Why then is recognition of this responsibility so long delayed in South Africa ?

Redaksioneel

STATUS, KWALIFIKASIE, VERANTWOORDELIKHEID

Die mediese laboratoiums tegnoloog is vandag die aspoester in die wetenskaplike wêreld, vernaamlik in die mediese gebied waar dit dikwels nog gevind word dat die tegnoloog beskou word as die „bottelwassers" in die laboratorium. Die verwarring wat daar heers tussen die werk van die mediese laboratoriums tegnoloog en die laboratoriums bediende is, in hedendaagse ingeligde omstandighede baie moeilik om te verstaan.

Die huidige mediese laboratoriums tegnoloog het 'n lang en moeilike opleiding deurgemaak en het ook 'n gesonde kennis opgedoen, in die menigde tekniek wat in die mediese laboratorium gebruik word. Sy opleiding het verstrek oor 'n tydperk van vyf jaar en gedurende die tyd het hy professionele eksamens van 'n besondere hoë gehalte afgelê— Selfs van so 'n hoë gehalte dat dit in sommige lande erken word as gelyk aan 'n gegradueerde standaard van onderwys.

Waarom dan is die mediese tegnoloog se opleiding beskou as minderwaardig in vergelyking met die gegradueerde wat met een of ander onderwerp van die wetenskap as hoofvak sy graad behaal en dan mediese laboratoriums tegnologie opneem ? Waarom is die onervare gegradueerde dikwels verkies voor die hoog-opgeleide en geoefende tegnoloog ?

Die moderne mediese laboratoriums tegnoloog is bekwaam om die organisasie en beheer van 'n kliniese laboratorium alleen te onderneem en word ook dikwels verwag om dit te doen. Die kliniese staf het 'n volle vertroue in sy werk en hy is beskou as 'n verantwoordelike lid van die hospitaal staf. Waarom dan word die erkenning van die verantwoordelikheid so lank vertraag in Suid-Afrika ?

Obituary

Professor M. VAN DEN ENDE,
M.B., Ch.B. (Cape Town), Ph.D. (Cantab.), F.R.S.S. Af.

It is with regret that the Cape Branch record the death of Professor M. van den Ende, Professor of Bacteriology and Dean of the Faculty of Medicine at the University of Cape Town. A good technician himself Professor van den Ende was always interested in the affairs of technicians. The Cape Branch owes much to his advice and encouragement in its early days ; and his loss will be felt by the many who knew and worked with him.

The Branch extends its sympathy to his family.

MICHAEL ROBERTS
(aged 21 years)

It is with deep regret that we record the death of Michael Roberts on 13th July, 1957, after a short illness.

Michael was educated at Kingswood College, Grahamstown, and the Observatory Boys' High School. After matriculating he became a trainee technician in the Pathology Department of the University of Cape Town.

He was a keen and meticulous worker. An excellent carpenter, he made several useful articles for the laboratory quite apart from the performance of his routine duties.

To his family we extend our heartfelt sympathy.

Reprint from *The Journal of Medical Laboratory Technology*

THE COOMBS TEST AND ITS APPLICATION IN PATHOLOGY

I. DUNSFORD

Regional Transfusion Centre, Sheffield
(From a lecture to the Liverpool Branch)

INTRODUCTION

When I was invited to address your branch, I deliberately chose this title because the anti-globulin reaction has been the main technique in the development of our knowledge of human blood groups ; a study of this technique contains interesting material both for the student technician, the chief technician and the pathologist ; furthermore its applications are becoming so wide that it is no longer confined to blood transfusion, and workers in many fields are using the anti-globulin technique as a routine.

Irrespective of the field of application, to understand the anti-globulin reaction it is necessary to have a knowledge of some of the basic facts of immunology. Agglutination of red cells, transfusion reactions, are manifestations of what is known as an antigen-antibody reaction, and so let us start with basic definitions. What is an antigen? An antigen is any substance which when introduced into the body of an animal lacking that substance causes the production of anti-substances known as antibodies. Antigens may be protein in nature and some carbohydrates are also antigenic, but irrespective of what type of antigen is used, the antibodies are always found in the serum of the immunised person, *i.e.*, one whose serum contains antibodies to a particular antigen. Taking the serum from such a person and studying in the laboratory its reactions with the antigen used for producing the antibody, is the science of serology. In serology, we classify antibodies according to the manner with which they react with antigens, so we have precipitins—the antigen being called a precipitogen, lysins—the antigen lysinogen, agglutinins—the antigen agglutinogen. These terms are simple ; precipitins causing precipitation of the antigen, lysins the dissolving of the antigen and the agglutinins the agglutination or specific clumping of the antigen. Two particular types of antibodies will now be defined that are not so well known. The first is the agglutinoids—these being antibodies which only give visible reactions with the corresponding antigen when the reaction takes place in protein or certain high molecular substances. The second group is the cryptagglutinoids which fail to give visible reactions with the corresponding reaction, irrespective of the type of suspending fluid

for the antigen, and they can only be detected by the anti-globulin technique. Another way of classifying antibodies broadly is based on the behaviour of the antibody with the antigen in isotonic saline which classifies them into two groups :—

(i) The complete antibodies or saline reacting antibodies.

(ii) The incomplete antibodies or saline non-agglutinating antibodies.

A complete antibody is one which gives an observable reaction with the corresponding antigen when this is suspended in isotonic saline. The incomplete antibody is one which fails to give an observable reaction with the corresponding antigen when this is suspended in isotonic saline —yet reaction has occurred as can be shown by various techniques, amongst them being the anti-globulin technique. In the complete antibodies we include lysins, precipitins, agglutinins and in the incomplete group are the agglutinoids and cryptagglutinoids.

Let us now consider the antigen-antibody reaction irrespective of the physical type of antibody. It is customary to consider serological reactions as occurring in two stages. This does not imply that the two stages are distinct for it is quite likely that the second stage starts before the first is entirely complete. The two stages are :—

Stage 1—The specific combination between specific fractions of the antibody and the corresponding factors of the antigen.

Stage 2—Observable reactions which may be agglutination, precipitation, etc.

The first stage occurs quickly and is possibly a chemical reaction whereas the second stage is slower and is a physical reaction depending on various factors, such as the medium in which the reaction has occurred. the quantities of antigen and antibody, the presence of complement, etc. Different factors are involved in different types of reactions; for example, complement is essential for lysins to be detected, but it is not essential for the reactions of agglutinins.

If we now reconsider the two broad types of antibodies, the complete and incomplete antibodies, it would appear that the incomplete antibodies are those which participate in stage 1, *i.e.*, specific combination, but are unable to complete stage 2 whilst the reaction is allowed to occur in saline. How do we know stage 1 has occurred ? How can we detect that the combination between antigen and antibody has occurred when there are no observable products ? Fig. 1 diagrammatically represents the two physical types of antibodies—the complete antibody as having two combining points—hence an alternative name, a bivalent antibody. The incomplete antibody, or univalent antibody is represented as having one combining site.

FIG. 1.—Types of Antibodies

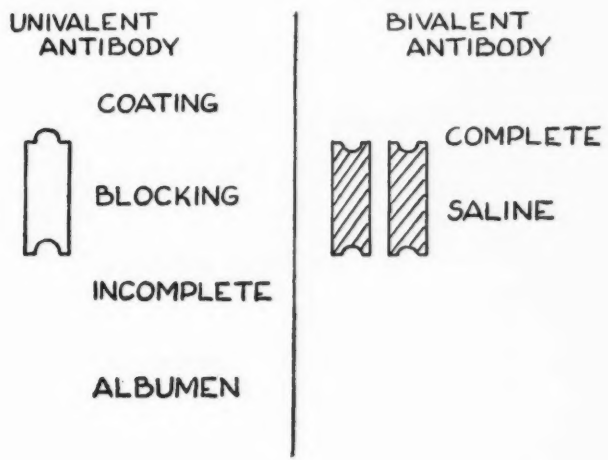
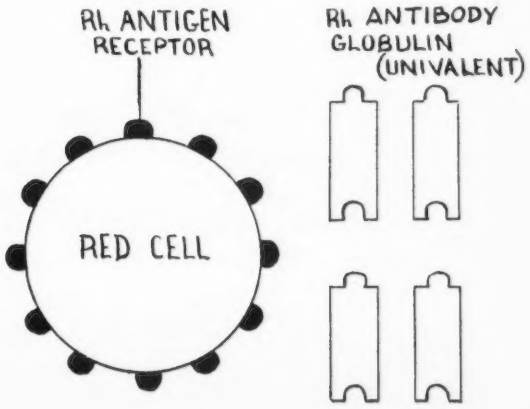


Fig. 2 diagrammatically shows the combining sites on a red cell for a Rhesus antigen and by the side are the incomplete antibodies to this particular antigen.

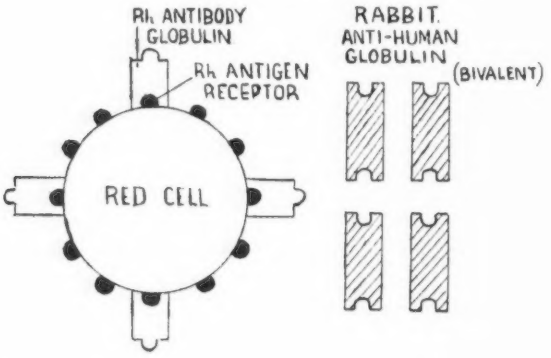
FIG. 2.—Coombs Test (A)



We must now consider the antibodies a little further. It has already been stated that the antibodies occur in the serum and it can be shown by fractionation that the antibodies occur particularly in the globulins. This applies to all species of animals. However, if we fractionate out the globulins from the sera of various animals whilst all give the chemical and physical reactions expected of globulins marked differences can be shown between these globulins by serological methods. If, for instance, we prepare a solution of goat globulins and a solution of guinea pig globulins and then inject these solutions into rabbits, *i.e.*, immunise them to these globulins, we find that the sera from the rabbits immunised with goat globulins agglutinates goat red cells but not guinea pig red cells, and the sera from the rabbits immunised with guinea pig globulin agglutinates guinea pig red cells, but not goat red cells. What is the explanation of these observations ? The answer is that no matter how purified our globulin fraction is, it contains substances specific to the species from which the globulins were obtained. These substances are the species specific antigens, and are integral parts of the antibody molecules. Therefore when we have an anti-Rh antibody in a human we know it occurs in the globulin fraction and any red cells taking up this antibody take up globulin, and since this globulin came from a human being it is human globulin.

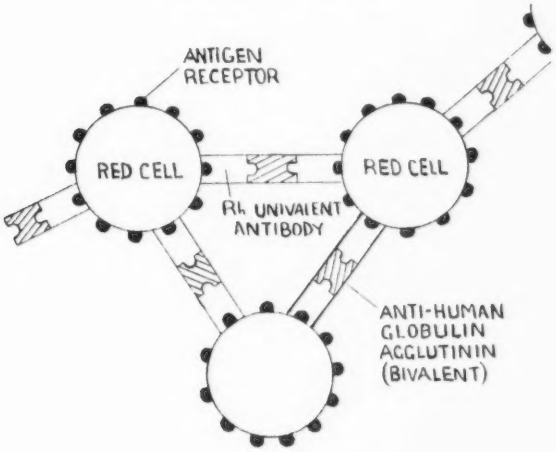
If we now consider Fig. 3 we see diagrammatically represented a red cell on which has been attached an incomplete univalent antibody.

FIG. 3.—Coombs Test (B)



On the same figure we see representation of the molecules of a complete—bivalent—rabbit anti-human globulin. Fig. 4 shows the effect of mixing these red cells, and we see why rabbit anti-human globulin will cause the agglutination of red cells on the surface of which are human antibodies, *i.e.*, human globulins.

FIG. 4.—Coombs Test (C)



This is the basis of the anti-globulin test and whilst antibodies are globulins, this test is not an anti-antibody test, it is an antigen-antibody reaction—the antigen being the globulin on the red cells and the antibody being the anti-species globulin.

HISTORY AND NOMENCLATURE

Many of you no doubt speak of the anti-globulin test as the Coombs test, inferring that it was discovered by Dr. Coombs, and if asked to date the discovery of this method would give 1945 as your answer. The anti-globulin test was first reported in 1908 by an Italian immunologist —Carlo Moreschi. In his papers Moreschi showed beyond doubt that red blood cells and bacteria, after reacting with the corresponding immune sera so diluted that they no longer agglutinated them, could be made to agglutinate rapidly if they were washed thoroughly and then exposed to an immune serum for the species of animal which provided the original immune serum. There is no doubt that Moreschi discovered the test and in 1953 Coombs delivered a lecture in Rome paying tribute to Moreschi for the discovery. Unfortunately between 1908 and 1945 this technique was not applied in routine serology and it was only in 1945 when we were faced with the problem of evolving methods for the detection of the then recently discovered incomplete Rhesus antibodies, that Coombs and his associates Race and Mourant rediscovered this technique. This illustrates magnificently the need for the wide reading of the past literature on a subject in addition to reading the latest publications.

It is obvious that to refer to this technique as the Coombs test is incorrect and indeed Dr. Coombs does not wish his name to be used, and prefers the technique to be known as the anti-globulin technique. This name has advantages in that by omitting the species, *e.g.*, anti-human globulin technique, it is a name that can be applied to the method irrespective of the anti-species globulins used. As will be shown later, the anti-globulin test has many applications and whilst the term anti-globulin test is a sound general name in the application of this technique in a particular study, it is essential, especially in research, to specify the species producing the anti-globulin as well as the species globulins used as antigen, *e.g.*, goat anti-rabbit globulin—the goat being the animal producing the anti-species globulin.

Another name that is used for the anti-globulin test is the "Development test." As various chemicals have the property of developing a photographic negative, so has the anti-globulin sera the property of developing the agglutination of coated cells. It is a good title in that it illustrates what the technique does, but gives no indication of the reagents used or what is developed.

We must now consider the terms 'Direct' and 'Indirect' anti-globulin test. The Direct test is the testing of cells—red blood cells or bacteria—with an anti-species globulin serum to determine whether the cells have a coating of antibodies. Coated cells, or sensitised cells as they are also called, give agglutination, *i.e.*, a positive Direct anti-globulin test. The Indirect anti-globulin test is a technique for investigating a serum for incomplete antibodies by determining its ability to coat red cells with antibody globulin. The Direct test is directly on cells whereas the Indirect goes indirectly to the cells by first exposing them to the immune serum under investigation.

Finally, comment should be made on the letters A.H.G. This laboratory abbreviation came into use about 1946–1947 to indicate anti-human globulin and it was therefore unfortunate that some haematologists a few years later decided to use the same abbreviation—A.H.G.—for anti-haemophiliac globulin. Therefore in a laboratory dealing with haematological as well as blood transfusion work the abbreviation A.H.G. can cause confusion and care is necessary.

APPLICATIONS

Having dealt with the principles and nomenclature of the anti-globulin test we will now consider its applications.

We will deal first with the field in which it has been mostly applied in the past ten years—blood transfusion. In 1945, Race and Wiener independently demonstrated the existence of incomplete forms of Rhesus antibodies. It was shown that these incomplete antibodies could cause

transfusion reactions and haemolytic disease of the newborn. Hence, with Coombs' rediscovery of the anti-globulin test this technique was applied in selecting compatible blood, investigation of transfusion reactions, and the investigation of cases of haemolytic disease of the newborn.

Let us first consider the anti-globulin technique for the detection of antibodies in comparison with other techniques. It will be seen from Table I that the anti-globulin test is the most efficient test of all for detecting antibodies to red cell antigens.

TABLE I

EFFICIENCY OF TECHNIQUES IN ANTIBODY DETECTION

Antibody	*Saline*	*Albumin*	*Enzymes*	*Anti-Globulin*
ANTI-A, ANTI-B	+	±	±	±
ANTI-Rh ..	∓	±	±	±
ANTI-KELL ..	∓	∓	∓	+
ANTI-MNSs ..	±	±	—	±
ANTI-DUFFY ..	∓	∓	∓	+
ANTI-KIDD ..	—	—	—	+

This knowledge is applied routinely in the examination of sera from expectant mothers to enable the forecasting of the possibility of haemolytic disease of the newborn. It is also applied routinely in the selection of compatible blood. As seen from Table II, recipients of blood are classified into two types.

TABLE II

CLASSIFICATION OF PATIENTS FOR BLOOD TRANSFUSION

Dangerous Recipients

Those who :—

(i) Have a history of previous blood transfusions.
(ii) Are parous women with an obstetric history suggestive of blood group incompatibility between mother and child.
(iii) Are suffering from haemolytic disease of the newborn.

Safer Recipients

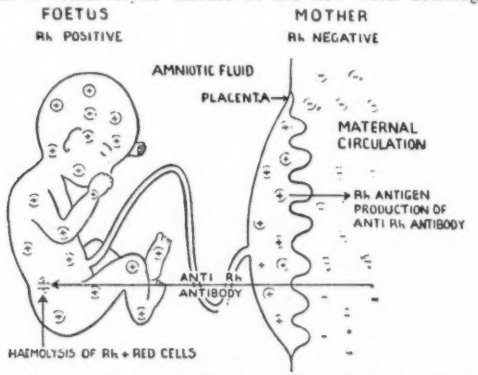
Those who :—

(i) Have no previous history of pregnancy or transfusion.
(ii) Are parous women, but whose obstetric history does not suggest any blood group incompatibility between mother and child.

For the Safer recipient a saline and albumin compatibility test is used, but for the Dangerous recipient an anti-globulin compatibility test is carried out. This is an indirect anti-globulin test using the patient's serum against the donor's cells or the anti-globulin compatibility test. This test is absolutely essential if the patient has had a previous transfusion as the patient may have produced anti-Duffy, anti-Kidd, etc., which are not detectable by the saline and albumin compatibility tests. The routine application of the anti-globulin test has greatly contributed to our knowledge of human blood groups and in turn to the science of human genetics. It is interesting to note that the Duffy blood group was discovered in 1950 by the anti-globulin test, and anti-Fy^a would not have been discovered until 1953 were it not for this test. This statement is based on the fact that the one and only saline reacting anti-Fy^a in the world was discovered here in Liverpool in 1953. Anti-Fy^b discovered in 1951 reacted strongly in albumin and so would have been discovered without the anti-globulin reaction, and hence would have been discovered first and this blood group system would not have been named Duffy.

Following the paper of Coombs *et al.* in 1945, the anti-globulin test was soon applied to the red cells of babies with haemolytic disease of the newborn, the aetiology of which is diagrammatically represented in Fig. 5.

FIG. 5.—Haemolytic disease of the new born aetiology



The mother is Rh Negative. The foetus is Rh Positive, having inherited this character from its father. Rh antigen (either as foetal red cells, or in soluble form via the amniotic fluid) passes from the foetus to the mother who responds by producing anti-Rh antibody. The antibody passes from the mother to the foetus whose Rh positive red cells it damages, leading to haemolysis and so to anaemia and jaundice. This process only takes place in a small minority of cases where an Rh negative mother has an Rh positive baby.

If the foetus possesses a red cell antigen inherited from its father, but foreign to the mother, and if the mother possesses an antibody to this antigen as a result of previous transfusion or pregnancies during which foetal red cells entered the maternal circulation, then the maternal

antibody traverses the placenta into the foetal circulation and attacks the foetal cells. Irrespective of whether the antibody in the mother's circulation is a saline agglutinating type or not, the immune antibody in the foetal circulation is of the non-saline agglutinating type and attaches itself to the child's cells. The child's cells are said to be coated or sensitized and this coating of antibody globulin is detected quite readily on cells from the cord blood. The direct anti-globulin test should be applied routinely on cord blood in all cases where the mother is Rhesus negative or where the mother is Rhesus positive but has a history of blood transfusion, or has had children which possibly suffered from haemolytic disease of the newborn, or her present child has jaundice or is anaemic. However, a warning must be given here that a negative direct anti-globulin test does not necessarily exclude haemolytic disease of the newborn, nor does a positive result by itself prove it. This will be dealt with more fully under interpretation of results.

It is opportune to mention here the application of the anti-globulin reaction in veterinary work. Haemolytic disease of the newborn has been proved to occur in various animals. Caroli and Bessis showed that newborn mules sometimes suffered from this condition due to anti-donkey antibodies being stimulated in the mare. The conditions have been demonstrated in racehorses and pigs, and has been produced experimentally in dogs, rabbits, cats and mice. In veterinary studies various types of anti-globulins have to be used such as rabbit anti-pig globulin, and providing due consideration is given to hetero-agglutinins and iso-agglutinins, the technique applied is the same as in human pathology. Certainly in the breeding of racehorses and pedigree pigs avoidance of maternal-foetal incompatibility is of great economic importance.

Coombs and his co-workers in 1946 stated, "It seems that the principle involved in the test described in this paper should be applicable to the detection of mild cases of auto-sensitization." In the same year Boorman *et al.* first reported that red cells from acquired haemolytic anaemia give a positive direct anti-globulin test whereas red cells from cases of congenital haemolytic anaemia do not. However, this test is full of pitfalls for the unwary as a positive direct anti-globulin test does not necessarily confirm the diagnosis of acquired haemolytic anaemia, nor does a negative test exclude such a diagnosis. The importance of not refrigerating or even cooling the patient's cells before testing and the necessity for serial dilutions of a selected anti-globulin serum will be dealt with when we consider interpretation of results.

Brief mention will now be made of the anti-globulin reaction being applied in bacteriology. In 1946, Morgan and Schutze described saline non-agglutinating antibodies in human sera to various Shigella organisms that could be demonstrated by an anti-globulin technique. In 1947 Griffiths reported the use of an anti-globulin technique to demonstrate incomplete antibodies in brucellosis. This was further confirmed by the work of Wagner and Kuhns in 1953. In 1955 Sol. Haberman re-examined the findings of Middlebrook and Dubos in tuberculosis. The

latter authors found that concentrated aqueous extracts of tubercle bacilli could be absorbed on the surface of human erythrocytes to give an agglutinable antigen to be used as an aid in the diagnosis of tuberculosis. Some doubts had been cast on the method of Middlebrook and Dubos since it yielded some negative results in proved active cases. Haberman examined the sera from 63 proved cases of T.B. and eight gave negative results, by the normal Middlebrook and Dubos method. However, the sera from these eight cases gave agglutination with sensitized cells with an anti-globulin technique. This proved the presence of incomplete antibodies and this, and the other few examples mentioned, demonstrate that incomplete antibodies are not confined to blood group serology, and all bacteriological serology must be reconsidered, and where suitable antigens and anti-globulin sera can be prepared, new techniques devised.

In 1949 Wiener applied the anti-globulin technique to determine the presence of globulins in solutions demonstrating small quantities in normal C.S.F. with increased amounts in such conditions as syphilis. To determine the presence of globulins in a solution, dilutions of the anti-globulin sera are mixed with the solution in varying dilutions. These mixtures are left together for at least 30 minutes and then each mixture is tested with sensitized cells, if agglutination occurs the original dilution of the sera contained no globulins whereas if no agglutination occurs

MICHROME STAINS AND REAGENTS

for Microscopy and Biology

Adonitol	Dextrin	Leishman Stain	Sudan Blue
Arabinose	Field's Stain	May-Grunwald Stain	Sudan 1, 2, 3, & 4
Asparagine	Gelatine	Methyl Red	Thioflavine, T & S
Auramine, O	Giemsa Stain	Peptone	Thionin
Azur 1, 2, B, C, & L	Glucose	Resazurin	Toluidine Blue
Azur 2 Eosin	Haematoxylin	Rosolic Acid	Urease
Beef Extract	Inositol	Sodium Desoxycholate	Vital Red
Brom Phenol Red	Janus Green, B	Sodium Taurocholate	Yeast Extract
Cedarwood Oil	Lacmoid	Sodium Thioglycollate	etc.

Water Soluble Indicators **Buffer Tablets** **Optoil:** synthetic, non-sticky immersion oil
Cristalite and Clearmount: Synthetic, colourless, neutral mountants
Microscopic Stains in tablet form
Water Testing Tablets and Outfits **Stains for fluorescence microscopy**
UNIFORM HIGH QUALITY, RELIABILITY AND IMMEDIATE DELIVERY
55-page Price List of Michrome Stains and Reagents available on request.

EDWARD GURR, LTD.

42 UPPER RICHMOND ROAD WEST, EAST SHEEN, LONDON, S.W.14

Telephone: Prospect 8051 & 7606 Cables: Michromlabs, Put., London

Now ready: **"Practical Manual of Medical and Biological Staining Techniques"** (2nd edition 460 pages; price 42/-) by Edward Gurr, author of "Microscopic Staining Techniques," Nos. 1, 2 and 3.

it shows the original solution contained globulins. This is illustrated in the following Tables.

TABLE III

SERIAL DILUTIONS ANTI-A 758

SERIAL DILUTIONS AHG 65	1:100	200	400	800	1600	3200	6400	12800	25600	51200	SENS: CELLS	NON-SENS:
1:2	—	—	+	+	+	+	+	+	+	+	+	+
4	—	—	+	+	+	+	+	+	+	+	+	+
8	—	—	—	—	+	+	+	+	+	+	+	—
16	—	—	—	—	—	+	+	+	+	+	+	—
32	—	—	—	—	—	—	+	+	+	+	+	—
64	—	—	—	—	—	—	—	—	—	—	+	—
128	—	—	—	—	—	—	—	—	—	—	+	—
256	—	—	—	—	—	—	—	—	—	—	+	—
512	—	—	—	—	—	—	—	—	—	—	—	—

In Table III we see the inhibiting effect of pooled human serum on a standard anti-human globulin sera. Table IV shows the effect of serum from a case of multiple myeloma with excess globulins against the same A.H.G.

TABLE IV

SERIAL DILUTIONS—H.S. SERUM—MULTIPLE MYELOMA

SERIAL DILUTIONS A.H.G. 65	1:100	200	400	800	1600	3200	6400	12800	25600	51200
1 : 2	—	—	—	—	+	+	+	+	+	+
4	—	—	—	—	—	+	+	+	+	+
8	—	—	—	—	—	—	—	+	+	+
16	—	—	—	—	—	—	—	—	—	—
32	—	—	—	—	—	—	—	—	—	—
64	—	—	—	—	—	—	—	—	—	—
128	—	—	—	—	—	—	—	—	—	—
256	—	—	—	—	—	—	—	—	—	—
512	—	—	—	—	—	—	—	—	—	—

The next two tables show a study of first a normal child's serum and then the serum from a child with agammaglobulinaemia.

TABLE V
SERIAL DILUTIONS—SERUM FROM NORMAL CHILD AGE 5 YEARS

SERIAL DILUTIONS A.H.G. 61	1:1	2	4	8	16	32	64	128	256	512	SENS: CELLS	NON-SENS:
1:1	—	—	—	—	—	—	+	+	+	+	+	—
2	—	—	—	—	—	—	+	+	+	+	+	—
4	—	—	—	—	—	—	+	+	+	+	+	—
8	—	—	—	—	—	—	—	—	—	+	+	—
16	—	—	—	—	—	—	—	—	—	—	+	—
32	—	—	—	—	—	—	—	—	—	—	+	—
64	—	—	—	—	—	—	—	—	—	—	+	—
128	—	—	—	—	—	—	—	—	—	—	+	—
256	—	—	—	—	—	—	—	—	—	—	+	—
512	—	—	—	—	—	—	—	—	—	—	+	—

TABLE VI
SERIAL DILUTIONS—E.J.M. SERUM—AGAMMAGLOBULINAEMIA

SERIAL DILUTIONS A.H.G. 61	1 : 1	2	4	8	16	32	64	128	256	512
1 : 1	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+	+	+
16	+	+	+	+	+	+	+	+	+	+
32	+	+	+	+	+	+	+	+	+	+
64	—	+	+	+	+	+	+	+	+	+
128	—	+	+	+	+	+	+	+	+	+
256	—	—	—	—	—	+	+	+	+	+
512	—	—	—	—	—	+	+	+	+	+

Indeed agammaglobulinaemia can be diagnosed with more certainty and with much greater speed serologically than by biochemical methods.

Detection of traces if globulins leads to the next application of the anti-globulin reaction, namely its application in forensic medicine. Allison and Morten (1953) claimed the anti-human globulin inhibition test to be very specific for identifying human blood or serum stains. They obtained absolute specificity with dried stains 10 years old and they found the test to work with mummified remains at least 5,000 years old. Andersen (1954) agreed with Allison and Morten that the test was more specific than the standard precipitin test, but doubted it was as sensitive. Andersen found chimpanzee serum and blood equal to human blood at inhibiting A.H.G., but found Rhesus monkey serum had no effect. So from forensic work into comparative zoology and the anti-globulin reaction can be applied in studying the relationship of various species by the ability of their sera to cross-react with various anti-species globulins.

Finally mention is made of the application of mixed agglutination techniques using anti-globulin sera to demonstrate the presence of platelet antibodies, platelet antigens, blood group antigens in the skin. The limits of application of the anti-globulin reaction has not been reached and indeed as shown by the work of Coombs and his school at Cambridge in building up lattices of coated cells, the anti-globulin technique may be a key to greater understanding of the physical forces of antigen/antibody reactions.

THE PREPARATION OF ANTI-GLOBULIN SERA

The principle is simple, namely the injecting of the globulins of one animal into another to produce anti-species globulin sera.

I will confine my comments to the most commonly used anti-globulin sera—anti-human globulin sera—and will start off with the animal for producing this reagent. The most commonly used animal is the rabbit and my experience, and that of Miss Ikin's at the Lister Institute, is that some varieties are better than others, Flemish and Chinchilla being good varieties, whilst Rex and Dutch are not so good. Kleozkowski in studying the ability of rabbits to produce antibodies postulated that the ability to produce high titre sera was genetically determined. At Sheffield we are now producing large quantities of anti-human globulin sera in goats. We feel the goat is the animal of choice in that it produces a better reagent in much larger quantities and, of course, is not prone to myxomatosis. Similarly sheep produce a good reagent, but horses fail to do so. However, if you are only requiring small quantities rabbits are ideal.

Having selected the animal let us now consider the antigen. Either human serum or plasma can be used, usually serum is preferred, but it must be from a group O person. The reason for this is that sera from

A, B or AB persons contain A and/or B substances in solution and the animal will produce anti-A and/or anti-B antibodies in addition to the desired anti-globulin antibodies. The next problem is whether to give the serum unchanged as the antigen, or whether to precipitate out the globulins and give these. I have tried both methods and find nothing to justify the precipitation of the globulins. Indeed this was Coombs' opinion in 1946 and still is, but since then various methods have been reported in the literature from alum precipitated globulin to eluates from red cells from cases of acquired haemolytic anaemia. As numerous as the methods of preparation of the antigen are the methods of introducing the antigen. Three rules must, however, be observed. Firstly, all intravenous injections must be preceded with an intra-peritoneal injection, less than 24 hours before the intravenous injection. This reduces the chance of anaphylactic shock. Secondly, when first exposing the animal to the antigen the more routes you use the greater the chances of immunization—we give intra-peritoneal, intravenous and intramuscular. Afterwards all that is necessary is an intraperitoneal injection to condition the animal and then an intravenous injection. The third point was first mentioned to me some years ago by Dr. Stratton of Manchester. The longer between the exposures to the antigens the greater the titre and the more specific the antibody. Multiple injections of an antigen increase the spectrum of activity of the serum, and therefore reduce the specificity. The method we have found most satisfactory in rabbits is to give 1·0 ml. serum intraperitoneally followed within 24 hours by 0·5 ml. serum diluted to 1·0 ml. with saline and given intravenously, and then 1·0 intramuscularly in each buttock. Repeat this one week later and bleed the animal 10–14 days later. If the animal has produced anti-human globulin antibodies with a titre of 1 : 256 or more, leave the animal 6–12 months on a full diet. Diet is important in antibody production and the animal must be given well-balanced protein feeds. At the end of the rest period give the animal one intra-peritoneal and one intravenous injection and bleed 10–14 days later. This will produce a very potent reagent.

STANDARDISATION OF THE REAGENT

Questions concerning the standardization of anti-globulin serum are very popular with examiners in the final Haematology and Blood Transfusion examinations of the Institute of Medical Laboratory Technology. The answers soon classify those who have carried out the procedures involved and those who have just read the theory. I will therefore try to deal with this to give maximum help to those preparing for their final examination.

First bleed the animal of about 5–10 mls. of blood and after allowing the blood to clot ring the sample to obtain the maximum yield of serum. Leave the clotted sample for 2–3 hours at 37° C. Separate the serum and centrifuge to obtain serum completely free from red cells. Inactivate the

complement by heating the serum at 56° C. for at least 30 minutes. Unless the complement is destroyed the reagent will haemolyse all types of human red cells, since at this stage the serum contains anti-human antibodies which with complement present, act as lysins.

The next stage is to determine whether or not the anti-human globulins are of sufficient titre to warrant processing the serum further. As shown in Table VII this is determined by preparing serial dilutions of the serum in saline from 1/1 to 1/1024 and testing these dilution against 50 per cent. suspensions of human red cells coated with incomplete Rhesus antibodies and human red cells free from such a coating.

TABLE VII

	Dilutions of Rabbit Serum in Saline										
	1	2	4	8	16	32	64	128	256	512	1024
RABBIT A (SUITABLE)											
50 per cent. Sensitized R.B.C.s	V	V	V	V	V	V	++	++	+	(+)	—
Non-Sensitized R.B.C.s	V	+	(+)	—	—	—	—	—	—	—	—
RABBIT B (UNSUITABLE)											
50 per cent. Sensitized R.B.C.s	V	++	++	++	+	+	(+)	—	—	—	—
Non-Sensitized R.B.C.s	V	++	+	+	(+)	—	—	—	—	—	—

The titre against the coated cells is the titre of the anti-human species antibodies and the anti-human globulin antibodies, whereas the titre against the normal cells is the titre of the anti-human species agglutinins. The titre against the sensitized cells must be at least 20 times that against the non-sensitized cells if the serum is worth processing—for example Rabbit A. If this difference is obtained bleed the animal for a larger quantity of blood and after separation and inactivation of the serum as before, the next step is to remove the anti-species antibodies but to leave the anti-human globulins. If this difference does not exist—Rabbit B— some prefer to destroy the animal whilst others rest it for six months and try again.

There are three methods of removing the anti-species antibodies—the first method is to dilute the serum so that the anti-species agglutinins are no longer active. This can only be done when the anti-species agglutinins are of low titre and limits the use of the reagent,—this will be dealt with later. The second method is to dilute the serum with saline—one part of serum to three parts of saline—and heat the diluted mixture at 70° C. for one hour. The dilution is to prevent coagulation and the heating destroys the anti-human species antibodies but not the anti-globulin. The reagent prepared in this manner is limited in its use in that it is diluted 1 in 4 before use. This will be dealt with more fully later.

However, it is a method that can be readily used in the smaller laboratory. The last method is removal of the anti-species agglutinins by absorption with well-washed human red cells. The cells used must be A_1B and O. Whilst O cells will remove the anti-species many rabbits have a naturally occurring anti-A type antibody and some have an anti-B type. Absorption with O cells only can leave these agglutinins so that the reagent readily agglutinates A_1 and A_1B or A_1B and B cells, even though they are not coated. The cells used for absorption must be washed completely free of all traces of plasma or serum—as will be shown later even 1 part of human serum in 4,000 parts of saline is sufficient to neutralize some dilutions of anti-human globulin serum. The washing is carried out at least six times and there must be at least 20 volumes of saline to one volume of packed red cells at each washing, with thorough mixing of cells and saline at each washing. Absorption is best carried out in two stages, first absorbing with an equal volume of washed O cells (or A_1B cells and then with an equal volume of wash A_1B (or O cells).

The next step is to determine the optimum dilution for use, *i.e.*, at what dilution will it give the strongest reactions with weakly sensitized red cells. However, before giving details of the method employed, I want to deal with the problem of zoning. It has been observed many times that when a serum containing an antibody is titred against the corresponding antigen, no agglutination occurs with the antibody undiluted or in low dilution, yet occurs readily with the antibody when it is further diluted.

TABLE VIII

	DILUTIONS OF ANTI-HUMAN GLOBULIN SERUM											
	1	2	4	8	16	32	64	128	256	512	1024	2048
WASHED SENSITIZED R.B.C.s	—	—	—	+	++	+++	++++	++++	++++	++	+	—
	PREZONE OR PROZONE			REACTION ZONE								POST-ZONE

A serum showing the behaviour as illustrated in Table VIII is said to zone. The lack of reactions with the antibody neat or in low dilution is known as the prezone or prozone, then comes the dilutions of the serum which give agglutination—the reaction zone, and lastly comes the dilutions of the serum where the antibody is so weak that it no longer agglutinates the red cells—the postzone. What are the causes of this phenomenon ?

Let us deal with the prozone area first. If to the unagglutinated mixture of rabbit anti-human globulin serum and wash sensitized cells we add more sensitized cells, then agglutination will occur. Alternatively

if we add a small amount of a very dilute solution of gamma globulin—to neutralize some of the rabbit anti-human globulin—agglutination occurs. Both these experiments point to an excess of antibody to antigen as being the cause of the prozone. However, if we take these unagglutinated cells from the prozone area, wash them well three times with saline and then test them with goat anti-rabbit globulin, agglutination occurs. This demonstrates that the red cells coated with human globulin have taken up rabbit incomplete anti-human globulin, which, being a rabbit globulin reacted with goat anti-rabbit globulin. Similarly it can be shown that the cells in the prozone area are coated in that they fail to react with an anti-globulin sera free from prozone, *i.e.*, a complete antibody. Another association between prozone and incomplete antibodies is the fact that prozone is usually present only when the animal has been repeatedly immunized. Repeated immunization tends to produce an incomplete or blocking form of antibody. This will be seen on the figures showing the two main types of anti-globulin sera, which appear later. The prozone is therefore certainly due to the quantitative aspect of the antigen and antibody reaction, and is probably also due to the blocking action of incomplete anti-globulin antibodies.

In the postzone area you may consider the negative reactions are due to the fact that the antibody is so dilute that it can no longer agglutinate the red cells. This is true for some dilutions, but I would like you to study this Table IX which demonstrates that the end point of a titration is dependent upon the amount of the antigen, *i.e.*, the strength of the red cells/used.

TABLE IX

Antigen Strength R.B.C. Suspension	Antibody Dilution 1	2	4	8	16	32	64	128	256	512	Titration Score
2%	V	++	++	+	+	(+)	GW	—	—	—	43
6%	V	V	++	+	(+)	—	—	—	—	—	36
12%	V	+	(+)	W	—	—	—	—	—	—	19
24%	+	(+)	GW	—	—	—	—	—	—	—	10
36%	+	GW	—	—	—	—	—	—	—	—	7
48%	GW	—	—	—	—	—	—	—	—	—	2
60%	—*	—	—	—	—	—	—	—	—	—	0
80%	—*	—	—	—	—	—	—	—	—	—	0
100%	—*	—	—	—	—	—	—	—	—	—	0

*These cells gave a positive direct antiglobulin reaction showing that the cells had absorbed the antibody.

This illustrates that the end point of a titration is directly proportional to the strength of the antigen used, *i.e.*, with excess red cells absorption of the antibody occurs. If we now consider the postzone area, it is evident that the end point will vary according to the strength of the cells used.

Finally, let us consider the reaction zone. It will be observed that the degree of reaction varies. Maximum agglutination occurs at these dilutions where there are optimum proportions of antigen to antibody.

Having considered the prozone phenomenon, we now proceed to the method of standardization. The first stage is the preparation of sensitized cells. For this a true incomplete weak anti-D—titre in albumin 1/16 to 1/32— is selected and serial dilutions are prepared 1 : 1 to 1 : 128. To each dilution is added a sufficient volume of 50 per cent. suspension of washed packed group O Rh positive cells, Each mixture is then incubated at 37° C. for 30 minutes. The cells from each mixture are then washed thoroughly three times with large volumes of saline to remove all traces of uncombined serum. This now gives a series of coated red cells of varying degrees of sensitization. At the same time as these cells are being washed cells of types A_1, B and O known not to be coated are washed. Each lot of washed cells is prepared as a 50 per cent. suspension of cells in saline. Whilst the cell washing is proceeding a large opal glass (20 in. by 20 in.) tile is marked out as follows :--

TABLE X

A.H.G. DILUTIONS		1	2	4	8	16	32	64	128	256	512	CELLS & SALINE
R.B.C.s SENSITIZED WITH ANTI-D DILUTED	1											
	2											
	4											
	8											
	16											
	32											
	64											
NON-SENSITIZED	A_1											
	B											
	O											

Prepare serial dilutions of the anti-human globulin serum (neat to 512 or more according to preliminary titration) so that 0·5 ml. of each dilution is available. Place one drop of each dilution of the A.H.G. in each of the ten vertical squares on the tile so that each dilution can be tested against progressively less sensitized cells and non-sensitized A_1, B and O cells. Moving from left to right add one drop of sensitized cells (then the nonsensitized cells) to each dilution of the A.H.G. Carefully rinse the pipette between each lot of cells. Mix each lot of cells and serum with a clean plastic rod. After standing for one minute—and this must be timed—rock the tile gently and allow to stand for a further two minutes. Repeat the rocking and standing and read in 5–7 minutes. Score the agglutination carefully and check all negative results with a ×10 hand lens.

The results are generally of two main types. Table XI shows the results with a newly immunized animal. The optimum dilution for detecting Rhesus antibodies would be between 1 : 8 and 1 : 16.

TABLE XI

A.H.G. DILUTIONS		1	2	4	8	16	32	64	128	256	512	CELLS & SALINE
R.B.C.S SENSITIZED WITH ANTI-D DILUTED	1	+++	+++	+++	+++	+++	++	+	(+)	(+)	—	—
	2	+++	+++	+++	+++	+++	+	(+)	(+)	GW	—	—
	4	++	++	++	++	+	+	(+)	(+)	—	—	—
	8	+	+	+	+	(+)	(+)	—	—	—	—	—
	16	(+)	(+)	(+)	(+)	GW	GW	—	—	—	—	—
	32	GW	GW	GW	GW	—	—	—	—	—	—	—
	64	—	—	—	—	—	—	—	—	—	—	—
NON-SENSITIZED	A_1	—	—	—	—	—	—	—	—	—	—	—
	B	—	—	—	—	—	—	—	—	—	—	—
	O	—	—	—	—	—	—	—	—	—	—	—

Table XII shows A.H.G. produced in an animal that has been repeatedly immunized and exhibits the prozone phenomenon.

TABLE XII

A.H.G. DILUTIONS		1	2	4	8	16	32	64	128	256	512	1024	2048	4096	8192	16384	SALINE AND R.B.C.s
R.B.C.s SENSITIZED WITH ANTI-D DILUTED	1	—	—	(+)	+	+	++	++	++	+++	+++	++	+	(+)	(+)	—	—
	2	—	—	—	(+)	+	+	++	++	+++	+++	+++	+	(+)	—	—	—
	4	—	—	—	—	—	(+)	+	++	++	++	++	+	(+)	—	—	—
	8	—	—	—	—	—	—	(+)	+	+	+	+	+	(+)	—	—	—
	16	—	—	—	—	—	—	—	—	(+)	(+)	(+)	(+)	(+)	—	—	—
	32	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	64	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
NON-SENSITIZED	A1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	B	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	O	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

The optimum dilution for this A.G.H. for detecting Rhesus antibodies would be about 1 : 1,000. The comparison of the reactions of the two main types shows the effect of hyper-immunization resulting in the prozone phenomenon which may be due to incomplete antibodies.

ANTI-GLOBULIN FRACTIONS

You may have observed that the optimum dilution for each A.H.G. is for the detection of cells coated with Rhesus antibodies. Coating antibodies may be of varying specificity, *e.g.*, anti-Kell, anti-Duffy, anti-Kidd, etc., or it may be the type of coating antibody found in acquired haemolytic anaemia. Experience shows that what may be the optimum dilution for one type of antibody may fail to detect another type of antibody. This is shown in Table XIII where the same A.H.G. was tested against a 50 per cent. suspension of cells from a case of acquired haemolytic anaemia and a 50 per cent. suspension of Rhesus positive cells coated with an incomplete anti-D.

TABLE XIII

A.H.G. DILUTIONS	1	2	4	8	16	32	64	128	256	512	1024	CELLS & SALINE
A.H.A. CELLS	+++	+++	++	+	(+)	—	—	—	—	—	—	—
Rh COATED CELLS	—	—	—	(+)	+	+	++	+++	++	+	—	—
NON-SENS. CELLS	—	—	—	—	—	—	—	—	—	—	—	—

The optimum dilution for detecting Rhesus antibodies was 1 : 128, but as this illustrates at this dilution it fails to react with the cells from the case of acquired haemolytic anaemia. What does this mean ? In 1950 Dacie demonstrated that normal human red cells, left in their own serum at 2–5° C. for a few hours and then washed in warm saline often gave a positive direct anti-globulin test. It was subsequently shown by Crawford *et al.* that this form of incomplete antibody—known as the Dacie cold incomplete antibody—is anti-H and is present in practically everybody's serum but O and A_2 persons' red cells are more likely to be coated than A_1 and A_1B. Refrigeration of blood samples from cases of anaemia, *i.e.*, where there is an excess of serum to red cells, will quite often cause a positive direct anti-globulin test. Therefore when investigating anaemias as to whether they are acquired haemolytic anaemias or not, it is essential to test red cells which have not been refrigerated. In 1950 there was available at least three types of coated cells—cells coated with specific incomplete antibodies, *e.g.*, anti-D, cells coated with the Dacie type cold incomplete antibody and cells from cases of acquired haemolytic anaemia. It was observed that the acquired haemolytic anaemias could be divided into two groups—those whose cells had a reaction zone with A.H.G. identical with red cells coated with Rhesus antibodies and the second type had red cells which had a different reaction zone giving optimum reactions with the A.H.G. undiluted or in low dilutions. The protocol illustrates the second type which is known as the cold auto-immune type whilst the former is known as the warm auto-immune type. Using the various types of cells mentioned, Dacie in 1951 demonstrated that an antiglobulin serum neutralized with small amounts of gamma globulins failed to react with Rh sensitized cells or with cells from patients with the warm type of acquired haemolytic anaemia. However, this anti-globulin serum would still react with red cells sensitized with the cold incomplete auto-antibodies or with red cells from the cold type of acquired haemolytic anaemia. This lead Dacie to the conclusion that Rhesus antibodies and antibodies from the warm type of haemolytic anaemias were gamma globulins whereas the cold type antibodies were probably not. Renton applying some of this knowledge was able by cross absorptions to fractionate an anti-globulin serum into two fractions. Subsequently work by Cutbush and others has shown that an anti-globulin serum may be a mixture of anti-human gamma globulins—probably against the gamma-2 fraction—anti-human alpha globulins and anti-human beta globulins and possibly more fractions yet to be discovered.

This now explains the reactions shown in Table XIII and it is of great importance in the technique of the anti-globulin test to realize that the A.H.G. may consist of both incomplete and complete antibodies against various globulin fractions. That even with optimum proportions of antigen to antibody one dilution may be suitable for detecting one antibody on red cells, *e.g.*, anti-D, but it may be quite unsuitable for detecting another type of antibody, *e.g.*, anti-Kell, on red cells. From

this it will be evident that a negative reaction with an anti-globulin serum so diluted that it will readily detect incomplete Rhesus antibodies only excludes Rhesus antibodies. Furthermore negative reactions with an anti-globulin serum at all dilutions does not exclude all antibodies since one antiglobulin serum may not contain all the fractions to detect all antibodies. Finally, in the removal of anti-species agglutinins, a technique which starts by diluting the serum may at the same time dilute out a particular anti-globulin antibody.

TECHNIQUE

We will deal first with the indirect anti-globulin test which is for the detection of incomplete antibodies against donor cells to avoid incompatible transfusions, or to detect antibodies during the ante-natal period, etc.

First the patient's serum, This should be free from cells and for the detection of incomplete anti-Lewis, and possibly some forms of anti-Kidd and immune anti-A, the serum should be less than 24 hours old. Whilst complement is not essential for the anti-globulin reaction, complement or some thermolabile serum constituent is essential for some antibodies, which are not gamma globulins, to sensitize red cells. This point must be remembered in selecting blood for patients with anti-Kidd or anti-Lewis in their serum.

Secondly the test cells or donor cells. These should be as fresh as possible and preferably should not have been refrigerated. In the case of donor cells this cannot be avoided, but the use of citrate in the pilot tubes reduces the difficulties due to cold incomplete antibodies since citrate 'fixes' complement components. Cold incomplete antibodies are not gamma globulins and work better in the presence of complement. The cells before exposure to the patient's serum must be well washed, preferably three times in at least twenty volumes of saline. The removal of serum or plasma from the cells gives better coating and the final reading is more clear cut, unwashed cells are the major cause of 'grainy' reactions, the name given to a doubtful anti-globulin reaction. Occasionally some workers use enzyme treated cells—papain, ficin or trypsin—for the direct anti-globulin test. However, this technique is difficult and readily gives rise to false positives.

Having obtained a cell-free sample of serum and well-washed cells, we come to the mixing. Remembering that an excess of cells to A.H.G. is required in the test, a sufficient quantity must be used. Secondly, in exposing the serum to the patient's cells absorption is required and not agglutination. As has previously been shown, absorption will occur more readily with packed cells than with a weak cell suspension and yet maximum absorption of globulin must occur. To achieve this, one volume of washed packed cells (or at least a 50 per cent. suspension)

is mixed with at least two volumes of the patient's serum. Normally the mixing is performed at room temperature, but in some cases, *e.g.*, in preparing blood for a case of acquired haemolytic anaemia of the cold type, the cells and serum are separately heated to 37° C. before mixing. After the mixing the mixture is incubated at 37° C. for a minimum of 30 minutes. In an emergency, incubation in a water bath at 37° C. for 10 minutes will detect all but the more feeble immune antibodies. This demonstrates how quickly stage 1 of the antigen-antibody reaction occurs, but as Wootten has shown, prolonged incubation up to six hours increases the intensity of the reaction of the coated cells with the A.H.G. Therefore whilst the cells take up sufficient globulins in a matter of minutes to give sufficient reaction with most anti-globulin sera, the longer the cells and serum mixture are left together the greater the uptake of antibody globulin.

The next stage of the indirect anti-globulin test is identical with the first stage of the direct anti-globulin test, namely the washing of the red cells free of all antibody globulin except that which is specifically combined with the antigen receptors on the red cells. This washing must be thorough, as even a trace of serum left with the cells will neutralize the anti-globulin serum, *i.e.*, diluted anti-globulin sera reacts with free globulin in preference to globulin attached to the red cells. This is possibly due to the fact that globulin attached to red cells has already used up half of its combining sites whereas free globulin has all its combining sites available. Table XIV shows the effects of traces of human serum and it will be seen that as little as one part of human serum in 4,000 parts of saline is sufficient to neutralize an equal volume of a diluted anti-human globulin serum.

TABLE XIV

EFFECTS OF TRACES OF HUMAN SERUM ON THE ANTI-HUMAN GLOBULIN REACTION

Dilutions of Human Serum in A.H.G.	*Rh + Coated Cells + A.H.G.*
1 in 1,000	—
1 in 2,000	—
1 in 4,000	—
1 in 6,000	GW
1 in 12,000	+
Control (No Serum)	++

The mixture of cells and serum should be in a 3 in. x $\frac{3}{8}$ in. tube and after centrifuging all the serum is removed with a fine pipette. The tube is then filled with isotonic saline, flushing the red cells from the bottom during the course of filling. The cells must be washed at least three times, each time using a large volume of saline, and the thorough mixing of the cells and the washing saline is essential.

Having washed the cells free of uncombined serum they are next prepared as at least a 50 per cent. suspension in fresh saline. It must be remembered that one of the causes of prozone is excess of antibody to antigen—the globulin attached to the red cell being the antigen. Therefore the use of too weak cell suspensions can lead to false negatives. Also when using a reagent issued by another laboratory the method must be identical with that used in the standardizing of the reagent. Any variation in technique demands re-standardization of the reagents.

The actual testing of the cells with A.H.G. is performed on an opal glass tile. Opal glass is selected because of it having a smoother surface, freer from small pits than any other type of white tile. The tile must be scrubbed with soap and hot water and then completely rinsed and dried just prior to use. This is essential to ensure that no traces of serum are present.

A tile technique is used in preference to a tube technique in that the A.H.G. is usually standardized to this method, the reactions are quicker and the results are easier to interpret. Originally in 1945 Coombs and his colleagues used a tube techique but changed to a tile method in 1946. Most leading authorities now prefer the tile method.

The next step is the preparation of the A.H.G. at the dilution recommended by the issuing laboratory. This dilution is usually the optimum for detecting Rhesus antibodies and may or may not detect other antibodies. It is advisable therefore to make a second dilution. The second dilution should be about 1 : 8 to 1 : 10 whilst the recommended dilution may be 1 : 20 ; to 1 : 100. These dilutions must be prepared in clean tubes, with a new pipette and teat ; every piece of equipment being scrupulously clean to ensure freedom from traces of human serum.

Having washed the cells and prepared the A.H.G. three drops of diluted A.H.G. are placed on the washed tile. On one drop place one drop of well-washed 50 per cent. red cells coated with a weak true incomplete Rhesus antibody—the positive control. On the next drop place one drop of 50 per cent. red cells known to be free from globulin coating —the negative control. On the last drop place one drop of the 50 per cent. suspension of washed cells and by the side of this place one drop of the 50 per cent. washed cells, without A.H.G., but with one drop of saline. The arrangement on the tile is as follows :—

POSITIVE CONTROL	NEGATIVE CONTROL	TEST	CELL SUSPENSION CONTROL

Repeat the process for the other dilution of the A.H.G. The suspension control is to check whether the washed cells agglutinate without A.H.G., *i.e.*, the patient's serum contained a complete antibody. The purpose of the positive control is obvious and the negative control is to check that the A.H.G. is free from anti-species agglutinins. The positive and negative control cells must be washed at the same time as the test cells. This is a control on the efficiency of the washing technique.

The next step is the mixing on the tile. Some people use the corner of a microscope slide using a clean corner for each mixing. There is a danger in this method since on mixing with glass on glass, according to the type of glass used, small amounts of silica become mixed with the red cells and will cause agglutination of the red cells, *i.e.*, a false positive. To avoid this each mixing should be performed with a clean plastic rod or a new swab stick can be used. As soon as the mixing is complete start a stop watch.

After mixing leave the tile on a level surface for one minute. Manipulation of the tile is a very important part of the technique, over rocking reducing the degree of agglutination. After one minute tilt the tile gently in one direction and bring it back to the horizontal and then place it back on the bench for a further two minutes. Pick up the tile, gently tilt the tile in one direction and then slowly rotate the tile. Leave the tile on the level surface and then repeat the rotating. Read the results in 5–7 minutes, checking all negative results with a x 10 hand lens. Doubtful results, the so-called 'grainy' results, will often disappear by the addition of one drop of saline whereas the true results are stable after such treatment.

INTERPRETATION

A negative or a positive result by itself means very little. Each reaction must be interpreted as only part of a serological syndrome—antagonism between an antigen and antibody—and if all is based on the antiglobulin reaction false results, negative or positive, can be very misleading. The interpretation of the results will be dealt with according to the application of the test.

In applying the test in the study of diagnosis of haemolytic disease of the newborn the diagnosis cannot be made with certainty by the anti-globulin test only. Haemolytic disease is the manifestation of incompatibility between mother and child's blood groups and for certain diagnosis the following criteria must be fulfilled :—

(*a*) The mother lacks a red cell antigen that the child (and its father) possesses.

(*b*) The mother's serum contains an antibody against her child's (and its father's) red cells.

(*c*) The child's red cells are coated with antibody and give a positive direct anti-globulin test.

Providing the first two tests are routinely carried out a false result in the anti-globulin test will be revealed. False negatives can be due to any of the following :—

(*a*) Using the A.H.G. at the wrong dilution. The direct anti-globulin test should be repeated using the A.H.G. in serial dilution.

(*b*) The baby is not newly born. Ideally the direct anti-globulin test should be performed on red cells from the cord blood. In haemolytic disease of the newborn the direct anti-globulin test may be positive only for a few days, but in other cases it may be still positive when the child is three weeks old. The testing of the mother's serum against the child's red cells using saline, albumin and anti-globulin techniques will overcome any difficulties due to this problem.

(*c*) The baby's cells may have been collected in saline or citrate solution. Postal transmission of baby's cells in saline or citrate to the laboratory is a common cause of false negative results due to the antibody being eluted from the red cells into the suspending fluid. All cells for testing must be from clotted specimens.

(*d*) The baby may be suffering from haemolytic disease of the newborn due to immune anti-A or immune anti-B. The diagnosis of H.D.N.B. due to immune alpha or beta is difficult and it is the experience of serologists that this condition can occur with a positive or negative direct anti-globulin test.

(*e*) The cells may not have been washed efficiently and traces of serum are neutralizing the reagent.

False positive direct anti-globulin tests on baby's red cells can be due to :—

(*a*) The passage of a Dacie type antibody from the mother across the placenta into the child's circulation. This will not harm the child but if the cord sample is refrigerated before testing a

positive Direct anti-globulin test may be found. Absence of other confirmatory serological findings on the mother's sample suggests a false positive and this is confirmed by finding the mother's cells to give a positive direct anti-globulin test and by finding a negative test on cells taken by heelprick from the child and testing the cells before refrigeration.

(*b*) Wharten's jelly attached to the red cells and their conglomerating being misread for agglutination. The mixing of the apparently washed cells with saline instead of A.H.G. gives the clue to this as these cells give an identical appearance to those tested with A.H.G. Repeating the test with cells obtained from a heel-prick or rewashing the cells from the cord sample in saline at 37° C. overcomes this type of difficulty.

Having given you the causes of false results in the anti-globulin test in diagnosing H.D.N.B., it is evident that it is essential to have a sample both from mother and child to diagnose this condition with certainty. Indeed incompatability occurs between two or more substances and incompatibility cannot be diagnosed on one sample only —the baby's.

Let us now consider the interpretation of results in haemolytic anaemia. The blood should be taken with a warm (37° C.), syringe, placed in a warm vial at 37° C. and kept at that temperature until clotted. Centrifuge the sample surrounded with water at 37° C. in the buckets, and then whilst still warm separate the serum from the clot. The direct anti-globulin test is performed immediately on washed cells from this sample. A negative test can be obtained even though the patient is suffering from acquired haemolytic anaemia, the negative result being due to :—

(*a*) The anti-globulin serum lacking fractions to detect the particular globulin coating. To avoid this use three different A.H.G. sera and do not use an anti-globulin that has been diluted as a part or whole of the process of removing anti-species agglutinins.

(*b*) The anti-globulin sera having been wrongly diluted and either the dilution is in the prezone or postzone. Serial dilutions from 1 : 1 to 1 : 512 of each A.H.G. sera will avoid this pitfall.

(*c*) The patient may be receiving cortisone therapy which sometimes temporary depresses the coating of the red cells.

(*d*) Traces of serum remaining through inefficient washing of the cells.

A positive direct anti-globulin test is not conclusive evidence of acquired haemolytic anaemia. The positive reaction could be due to :—

(*a*) The patient's cells being coated with an incomplete Dacie auto-antibody. Generally this is avoided by the procedure

outlined for the collection of the sample. Occasionally a normal healthy person is found with a positive direct anti-globulin test due to this type of auto-antibody. To avoid interference from this it becomes necessary to take the blood from an ear prick or finger prick direct into warm (40° C.) sodium citrate solution.

(*b*) The patient may have received an incompatible transfusion. For 24 to 48 hours after an incompatible transfusion a positive direct anti-globulin test can be found. This is due to the donor cells having taken up the patient's antibody *in vivo*. This can occur without clinical evidence of a reaction. If possible avoid testing a sample within 48 hours of a transfusion.

Other serological findings such as the presence of auto- and pan-agglutinins, etc., are confirmatory evidence of acquired haemolytic anaemia together with positive haematological findings.

Finally let us consider the interpretation of the results of the indirect anti-globulin test in the compatibility test or in the examination of sera from expectant mothers. The comments on false negatives due to wrong dilution of the A.H.G., inadequate washing, apply equally well here as in other fields of application, these remarks will be confined to the interpretation of the positive results. When a positive result is obtained against donor cells after they have been exposed to the patient's serum, then those cells without exposure to the serum must not give a positive direct anti-globulin test with the same A.H.G. at the same dilution. If the donor cells do give a positive direct anti-globulin then the result from testing the patient's serum against the same donor cells was false. The type of false result can be caused by a Dacie type incomplete antibody and can also occur with cells that have been frozen in glycerol and trisodium citrate. During the recovery of the cells from the frozen state an uptake of silica may occur and this may be the cause of this type of false positive result.

CONCLUSION

From my remarks this evening it will be seen that the anti-globulin test challenges the skill of the technologist, it is the tool *par excellence* of the serologist, and the limits of its application are yet to be found. It is regrettable that the work of Moreschi was so long forgotten, but fortunately Coombs' rediscovery of the anti-globulin test has opened up wide vistas in the realms of medical science.

REFERENCES

ALLISON, A. C., & MORTEN, J. A. (1953). "Species specificity of the inhibition of anti-globulin sera." *J. clin. Path.* **6.** 314.

ANDERSON, J. R. (1954). "Application of anti-globulin reaction to blood stains in determination of animal species." *Am. J. clin. Path.* **24.** 920.

BARRIE, J., & KREIGER, V. I. (1954). "A note on decrease of avidity of the Coombs reaction by gradual elution of Rh antibodies from the sensitized cells." *Med. J. Austral.* 247.

BOORMAN, K. E., *et al.* (1946). "Haemolytic Icterus (acholuric jaundice) congenital and acquired." *Lancet.* **(i).** 812.

BOORMAN, K. E., & DODD, B. E. (1946). "An incomplete form of agglutinin." *Nature.* **158.** 589.

CAROLI, J., & BESSIS, M. (1947). "Recherches sur la cause de l'ictere grave familial des muletons." *Rev. d'Hem.* **2.** 207.

CLEARKEN, K. P. (1952). "Coombs test in acquired haemolytic anaemia." *Lancet.* **(i).** 183.

COOMBS, R. R. A., *et al.* (1945). "Detection of weak and incomplete Rh agglutinins. A new test." *Lancet.* **(ii).** 15.

COOMBS, R. R. A., *et al.* (1946). "*In vivo* isosensitization of red cells in babies with haemolytic disease." *Lancet.* **(i).** 264.

COOMBS, R. R. A., *et al.* (1948). "Haemolytic disease of newborn foals due to iso-immunization of pregnancy." *J. Hyg.* (*Camb.*). **46.** 403.

COOMBS, R. R. A., *et al.* (1956). "Specific mixed agglutination, mixed erythrocyte-platelet anti-globulin reaction for the detection of platelet antibodies." *Brit. J. Haemat.* **(ii).** 84.

COOMBS, R. R. A. (1949). "Discussion. Haemolytic disease of the newborn." *Proc. Roy. Soc. Med.* **43.** 347.

CRAWFORD, H., *et al.* (1953). "Specificity of incomplete 'cold' antibody in human serum." *Lancet.* **(i).** 566.

CRAWFORD, H., & MOLLISON, P. L. (1951). "Demonstration of multiple antibodies in anti-globulin sera." *Lancet.* **(ii).** 955.

DACIE, J. V. (1950). "Occurrences in normal human sera of incomplete forms of cold auto-antibodies." *Nature.* **166.** 36.

DACIE, J. V. (1953). "Acquired haemolytic anaemias with special reference to the anti-human globulin test." *Blood.* **8.** 813.

DACIE, J. V. (1951). "Differences in behaviour of sensitized red cells to agglutination by anti-globulin sera." *Lancet.* **(ii).** 954.

DUNSFORD, I., *et al.* (1951). "The Coombs test." *J. med. Lab. Techn.* **9.** 137.

ELLIS, E. (1952). "A slide test procedure using anti-human globulin serum (Coombs serum)." *Am. J. med. Techn.* **18.** 229.

EMERSEN, *et al.* (1951).. "The production of potent anti-human globulin (Coombs reagent) in rabbits immunized with serum adjuvant mixtures." *J. Immunol.* **66.** 323.

EVANS, R. S., *et al.* (1947). "Demonstration of antibodies in acquired haemolytic anaemia with anti-human globulin serum." *Proc. Soc. Exp. Biol. & Med.* **64.** 372.

GRIFFITHS, J. J. (1947). *Publ. Health Rep.* **62.** 865.

HABER, J. M. (1954). "Indirect Coombs incompatibility." *Am. J. Med. Techn.* **20.** 3.

HABERMAN, Sol., *et al.* (1955). "Detecting Tuberculosis Antibodies." *Brit. med. Journ.* 620.

HACKETT, E. (1950). "Coombs test in acute acquired haemolytic anaemia." *Lancet.* **(i).** 998.

HACKET, E. (1950). "The Coombs test and guinea pig complement." *Nature.* **165.** 764.

HARE, R. L., *et al.* (1954). "The anti-globulin (Coombs) test as an aid in the diagnosis of haemolytic syndromes." *J. Lab. & Clin. Med.* **43.** 867.

HILL, J. M., *et al.* (1948). "Haemolytic Rh immune globulins : evidence for a possible third order of antibodies incapable of agglutination or blocking." *Blood.* **3.** Suppl. 2. 80.

HILL, J. M., & HABERMAN, SOL. (1954). "The Coombs (anti-globulin test). Indication and technics." *Am. J. clin. Path.* **24.** 305.

HOLLANDER, L. (1952). "Incomplete cold agglutinins as a cause of a positive anti-globulin test in a neo-nate." *Helv. paediat. Acta.* **7.** 512.

HUBINOT, P. O., *et al.* (1950). "Remarkques sur la reaction de Coombs. II. Variations de la reaction indirecte." *Rev. d'Hem.* **5.** 3.

JAKOBOWICZ, *et al.* (1950). "Occurrence of unusual positive Coombs reactions and 'M' factors in the blood of a mother and her first baby." *Nature.* **165.** 158.

JACOBOWICZ, R., *et al.* (1948). "The value of the Coombs test in detection of iso-sensitization of the newborn." *Med. J. Austral.* 143.

KOMNINOS, Z. D., & AKSOY, M. (1955). "Production of Coombs serum by injections of auto-antibodies into rabbits." *Am. J. clin. Path.* **25.** 967.

LEONE, CHARLES A. (1952). "Effect of multiple injections of antigen upon the specificity of anti-sera." *J. Immunology.* **69.** 285.

KLECZKOWSKI, A. (1939). *Ztchr. Immunitatsforch.* **95.** 218'

LIU, CHI KING, & EVANS, R. S. (1952). "Preparation of anti-rabbit globulin guinea pig serum." *Proc. Soc. Exp. Biol. (N.Y.).* **79.** 194.

LOUTIT, J. F., & MOLLISON, P. (1946). "Haemolytic Icterus (acholuric jaundice,) Congenital and Acquired. *J. Path. & Bact.* **58.** 711.

MCNEILL, C., & TRENTELMAN, E. F. (1951). "Detection of antibodies by polyvinyl-pyrrolidone. II. Comparison of sensitivity of anti-human globulin and PVP." *Proc. Soc. Exp. Bio. & Med.* **78.** 674.

MENOLASINO, N. J., & DAVIDSON, I. (1954). "A simplified method of preparation of anti-human globulin (Coombs) serum." *Am. J. clin. Path.* **24.** 1205.

MONTGOMERY, C. M. (1946). *Med. J. Austral.* **1.** 693.

MORESCHI, C. (1907). "Bescheunigung und Verstarkung der bakterienagglutination durch anticiweissera." *Centralbl. f. Bakt.* **46.** 456.

MORESCHI, C. (1907). *Centralbl. f. Bakt.* **46.** 49.

MORGAN, W. T. J., & SCHUTZE, H. (1946). *Brit. J. exp. Biol.* **27.** 286.

MOLLISON, P. L., & CUTBUTSH, M. (1955). "Use of isotope-labelled red cells to demonstrate incompatibility *in vivo*." *Lancet.* (i). 1290.

MILGROM, F., *et al.* (1956). "Preparation of anti-globulin sera." *Nature.* **177.** 329.

RENTON, P. H. (1952). "Separation of Coombs reagent into two fractions." *Nature.* **169.** 329.

ROSENFIELD, R. E., *et al.* (1951). "The anti-globulin test. Technic and practical applications." *Am. J. clin. Path.* **21.** 301.

SELWYN, J. G., & ALEXANDER, S. S. (1951). "A positive Coombs reaction in pernicious anaemia." *Brit. med. Journ.* (i). 564.

SINGER, K., & MOTULSKY, A. G. (1948). "The developing (Coombs) test in spherocytic (haemolytic) anaemias ; its significance for the patho-physiology of spherocytosis and splenic hemolysis." *J. Lab. & clin. Med.* **33.** 1197.

SINGH, J., *et al.* (1952). "Human plasma as antigen in the preparation of precipitin serum." *Nature.* **169.** 4291.

SPAET, T. H., & KINSELL, B. F. (1953). "Positive Coombs test due to Dacie type incomplete antibody." *J. Lab. & clin. Med.* **42.** 205.

STRATTON, F., & JONES, A. R. (1955). "The reactions between normal human red cells and anti-globulin (Coombs) serum." *J. Immunology.* **75.** 423.

STRATTON, F., & RENTON, P. H. (1955). "Effect of crystalloid solutions prepared in glass bottles on human red cells." *Nature.* **175.** 727.

STRATTON, F. (1956). "Production of human anti-globulin reagent in sheep" *Nature.* **178.** 214.

STURGEON, P. (1954). "The nature of 'complete' Rh anti-sera as revealed by anti-globulin augmentation." *J. Immunol.* **73.** 212.

STURGEON, P. (1947). "A new antibody in serum of patients with acquired haemolytic anaemia." *Science*. **106.** 292.
WAGLEY, P. F., *et al.* (1948). "The spleen as a source of a substance causing agglutination of the red blood cells of certain patients with acquired haemolytic jaundice by an anti-human serum rabbit serum (Coombs serum)." *J. Lab. & clin. Med.* **33.** 1197.
WAGNER, B. M., & KUHNS, D. M. (1953). "Coombs type antibodies (anti-globulin) in Brucellosis." *Am. J. clin. Path.* **23.** 185.
WASASTJERNA, CURT, *et al.* (1954). "Intravascular agglutination of red cells." *J. Lab. & clin. Med.* **43.** 98.
WILSON, M. M., *et al.* (1951). "Coombs test in Brucellosis." *Lancet*. 17 Nov.
WOOTON, I. D. P. (1951). "Factors affecting the speed of agglutination in the Coombs test." *J. clin. Path.* **4.** 296.
WOOTON, I. D. P. (1950). "Production of Coombs' serum." *Nature*. **165.** 730.
WIENER, A. S., & GORDON, E. B. (1953). "Quantitative test for antibody globulin coating human red blood cells." *Am. J. clin. Path.* **23.** 429.
WIENER, A. S. (1949). "A new serological test (inhibition test) for human serum globulin." *Proc. Soc. exp. Biol. & Med.* **71.** 96.
WRIGHT, C. S., *et al.* (1951). "Studies of hemagglutinins in hereditary spherocytosis and in acquired haemolytic anaemia." *J. Lab. & clin. Med.* **37.** 165.
UNGER, L. J. (1951). "A method for detecting Rh antibodies in extremely low titer." *J. Lab. & clin. Med.* **37.** 825.

(*These references cover a much wider field than the subject matter of this lecture, and are given for the assistance of the more advanced worker on the subject.*—ED.)

Reprinted from *The Journal of Medical Laboratory Technology* by kind permission of the author and of The Institute of Medical Laboratory Technology, 9 Harley Street, London, W.1.

MEGALOBLASTIC ANAEMIAS

(*Synopsis of a lecture given by Prof. Adams, Professor of Medicine, Medical School, Durban, to the Natal Branch of the Society on 13th June*, 1957.)

Professor Adams opened his lecture by stressing the important part played by the technologist in the diagnosis of megaloblastic anaemia, depending as it does on microscopic examination of blood and marrow smears.

With the aid of the projector Prof. Adams illustrated the essential differences between normoblasts and megaloblasts, mentioning that once one has seen a film containing megaloblasts the picture is not easily forgotten. The differences between the blood picture of macrocytic anaemia and iron deficiency were described, megaloblastic anaemia giving a macrocytic picture. The speaker stated that if only *one* megaloblast is seen in a peripheral blood film, then it can be said with certainty that the patient is suffering from megaloblastic anaemia. These diagnostic cells may not always be seen in blood films, however, and films from the bone-marrow will confirm the diagnosis.

Deficiency of folic acid or Vit. B.12 is generally considered the cause of megaloblastic anaemia which not only affects the red cells but also the leucocytes, and bizarre forms are seen, the metamyelocytes being particularly affected to give giant forms.

A large number of megaloblastic anaemias are known. Pernicious anaemia of the white races is a classical example but it is uncommon in Africans. Pernicious anaemia is associated with atrophy of the gastric mucosa and no free HCl is demonstrable in the gastric juices of people suffering from this form of anaemia. The response to Vit. B.12 treatment is dramatic and although the patient responds also to folic acid there are side effects associated with this treatment such as degenerative changes in the spinal cord.

Another common megaloblastic anaemia is that associated with pregnancy. This form is more common in people living under poor conditions and deficient diet is probably the cause. This type responds satisfactorily to folic acid treatment.

A patient with pernicious anaemia must have continuous treatment whereas in a person suffering from anaemia of pregnancy the treatment is discontinued when the blood picture returns to normal. The differentiation of these anaemias is therefore most important.

In explaining the physiological aspect of pernicious anaemia, Prof. Adams quoted Castle's hypothesis where the extrinsic or food factor combines with the intrinsic factor found in gastric juice to produce the haemopoietic principle which brings about the maturation of blood cells.

Patients suffering from pernicious anaemia lack the intrinsic factor. Because of this deficiency these patients respond to Vit. B.12 only when given intramuscularly.

The speaker went on to describe other varieties, such as megaloblastic anaemia caused by certain species of tape-worm, the type associated with Steatorrhoea and post-operative anaemia associated with gastrectomy.

He mentioned the work of Watson on experimentally produced megaloblastic anaemia in animals, and Foy and Kondi's treatment of megaloblastic anaemia with antibiotics. Vit. B.12 is produced by bacteria in the lower gut but it is not absorbed. It can be extracted from the faeces of patients with megaloblastic anaemia and used for their treatment.

After the lecture members were invited to a demonstration of slides and to see a method for the determination of Vit. B.12 in serum. The method demonstrated was a microbiological one using a test organism, the alga euglena Z, which needs light and Vit. B.12 for multiplication. When the light is constant, its growth is proportional to the concen-

tration of Vit. B.12 present, and the growth produced by known quantities of Vit. B.12 can be compared with that in various dilutions of unknown sera.

Prof. Adams said that radio-active isotopes would soon be used at the Medical School to trace Vit. B.12 given to patients and so enable workers to determine the amount absorbed.

The speaker answered numerous questions put to him and was thanked on the members' behalf by Mr. A. Scott, Chairman of the Natal Branch.

J.R.H.

THE TEACHING OF BIOCHEMISTRY TO APPRENTICES

W. M. POLITZER

South African Institute for Medical Research

With the increasing importance of biochemistry it becomes necessary to give consideration to the curriculum of the apprentice. It is necessary to equip the student-apprentice thoroughly as an accurate result is frequently a matter of life or death to the patient.

No general scheme for training apprentices has been adopted as yet. It is vital that a programme of training be drawn up and that there should be uniformity in teaching and standard of knowledge throughout the country.

When selecting applicants for the course preference should be given to those matriculants who, besides mathematics, have taken physics and chemistry, or at least one science subject. This background will given them a good start.

The syllabus should begin with physics and follow on with inorganic, organic and physical chemistry. If all these preliminary subjects are left out of the syllabus—as has been suggested—the student apprentice has to learn biochemistry without this preparation, and he will be handicapped by the lack of knowledge of these basic sciences. This, of course, applies to all branches of pathology. He will do his work like a machine without understanding the principle behind the test he is doing. This is all wrong. He must know the basic laws as biochemistry is the application of these laws to the living body. Only with this background will he become interested in biochemistry and be able to adapt himself to new methods and techniques.

GENERAL TRAINING

His training in physics should be of the standard of matriculation physics. His inorganic chemistry training should be based on the periodic classification of the elements and the main emphasis should be on the 20 elements present in the body. In his organic chemistry training the classification of the properties of compounds depending on their structure should be emphasized. In their practical work the stress in both inorganic and organic chemistry should be laid on quantitative estimations.

It is often said that physical chemistry need not be taught to these students, but progress has been tremendous in this field (and in biophysics) since the last war. Advances made in photo-electric colorimetry, flame photometry, chromatography and radio-active isotopes have revolutionised laboratory techniques. It is therefore essential that this subject be taught.

It is vital that the whole programme of training should be so planned that it is a continuous building-up process so that the biochemistry training of the apprentice can be based on the knowledge he has gained in his first and second year. All his teachers must be thoroughly acquainted with the work that will be required of the student apprentice in the biochemistry department and co-operation between them and the head of the biochemistry department is of great importance. In fact, it would be advisable for the teachers to be chosen from the laboratory staff as they alone know what will be required of the student.

When the apprentice-student starts his third year with biochemistry he must have learnt the principles of visual and photo-electric colorimetry, how to pipet and calibrate glassware, what molecular and equivalent weight and milliequivalent are, what standard solutions, buffers and indicators are, what pH, osmotic pressure and a colloidal solution are, how all the different titrations are carried out including back titration and oxidation—reduction titration and how to make up more complicated solutions ; and he must know the properties of carbohydrates, fats and proteins.

When learning biochemistry he must be taught the principle of each estimation and he must know how to work out his own results from first principles. Incomplete comprehension of the principle of a test may be dangerous as short-cuts may be used with disastrous consequences.

Whilst 20 years ago most of the tests were done on a qualitative or semi-qualitative basis, most of the estimations are now done quantitatively, and accurate measurements must be carried out. Semimicro and micromethods are rapidly becoming standard practice and accurate estimations on very small quantities are much more difficult. Absolute

accuracy is essential as even a small error will be greatly magnified. The apprentice must be made to realise that an inaccurate result may have serious consequences for the patient and be the cause of his receiving completely wrong treatment, or leading to his death.

To summarise, the course for the diploma in technology must be both academic and practical, in order to create and operate an efficient service. A high standard of teaching is required, and in order to meet new needs continued consultation and co-operation should be maintained between teachers and laboratories.

The apprentice must realise that he is not just learning to do a "job" but that he has an enormous responsibility to the patient.

THE READERS' FORUM

The Editor,

Sir,

The article by Wikeley and Hart (this journal, **3**, 13), on the teaching of medical laboratory technology is, it is felt, altogether sound and their proposals are highly commendable.

The suggestion concerning bursaries awarded by employers of medical laboratory technologists is very practical, but it is believed that provision should be made for the holders of bursaries to be relieved of their suggested obligations to the bursars by making the bursary convertible into a loan repayable with interest at the discretion of the bursars. This would enable persons unable (for social, medical, or other reasons) to work for their bursars to discharge their obligation.

It is felt that the twelve weeks of Chemistry and Physics on the six months general introductory course should not be regarded as a "Refresher course," since many student medical laboratory technologists have little or no knowledge of either subject, and would, therefore, have to be instructed in the fundamentals of both.

There will, no doubt, be many who will disagree with the proposed time allotments for the various suggested divisions of the so-called professional course, but basically it is believed that there can be little founded criticism. Such small deviations as might be advisable from these proposed divisions could be agreed upon by general discussion among those concerned nearer the time, when and if the whole-time course becomes a reality.

Yours faithfully,

PETER N. BUCK.

The Editor,

Dear Sir,

It appears that my letter which appeared in the last issue of this Journal (Vol. 3, No. 3) has been misunderstood in some quarters and I must hasten to correct this false impression which has been gained, largely I think because of the time lapse between letters on the same subject. My object was to show that the course of training at the Natal Technical College was the first course organised subsequent to the publication of Government Notice 2043 in 1949.

If I have given the impression that I consider that any course which was running before that date was not an adequate course of training I am extremely sorry, such was not my intention. Perhaps our pardonable pride in having assisted in organising, in Natal, a course designed to meet the needs of the regulations and open to all workers in approved laboratories, leads to undue stress on this point ?

Yours faithfully

G. W. WIKELEY.

The Editor,

Sir,

It may be of interest to your readers to know that the Institute of Medical Laboratory Technology (of Great Britain) has recently published a Code of Professional Conduct (Gazette, 1957). Although the Society is largely modelled on the Institute, the Code is not altogether applicable, due to differences in grades of technologists and in types of employers. However, the items of the Code are worthy of note. It states, in effect, that technologists shall exercise a proper degree of professional skill and care, shall not disclose confidential information to unauthorized persons, shall take reasonable measures to maintain and improve professional skill and knowledge, shall instruct and encourage relevant study by students, shall always act in good faith towards professional associates, shall not accept or perform tests outside the jurisdiction of the employer, shall not assume ability to diagnose or treat disease, shall not perform tests for and disclose facts to an unqualified practitioner.

The Institute has, in addition, laid down a mechanism for the implementation of the Code.

It is strongly felt that two items in the Code would bear enforcement occasionally in this Society. One is that which states that technologists "shall take such measures as may be reasonable to maintain and improve their professional skill and knowledge." Happily, many qualified technologists fully realize the necessity for keeping abreast of advances,

particularly in their chosen sphere, but there are a regrettably large number who, having qualified, never again voluntarily open a text-book or relevant technical publication of any sort. The other item states that among prohibited acts is that of any technologist "in holding himself out as a person who by training and experience is professionally qualified to diagnose or treat disease in man or animals." There can be few technologists indeed, who, at some time or another, have not been asked for an opinion by a medical practitioner. If often appears so natural and innocuous but it is not proper for technologists to express opinions as to the application of results. These questions are often, but not always, in connection with borderline cases : a blood urea of 38 mgms.%, a faecal fat content of 28%, a slightly icteric serum, a moderately heavy growth of *Streptococcus pneumoniae* in a sputum with other organisms, a few *Giardia lamblia* is a specimen of faeces, one positive and one negative serological test for syphilis. In all such and similar cases, a question requesting an opinion should be met by some such answer as "I am not in a position to answer the question," or "I'm sorry, I can't say."

It is believed that the ethical standard of technologists in this Society is high, but it might be well for consideration to be given to the possibility of adopting a similar Code with an eye to the fortunately rare exceptional case.

Yours faithfully,

PETER N. BUCK.

Reference :

Gaz. Inst. Med. Lab. Tech. (1957). **4,** 78.

VACANCIES

THE SOUTH AFRICAN COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH PNEUMOCONIOSIS RESEARCH UNIT AT THE SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH, JOHANNESBURG

Applications are invited for senior medical technologists in the Department of Histopathology of the Pneumoconiosis Research Unit. The salary scales are—

£810×42—£1,020 and £1,020×60—£1,200

in addition a cost of living allowance of £234 per annum is payable to married men.

Membership of the Staff Provident Fund is compulsory and for this purpose a medical certificate of sound health is required.

The minimum qualification required is A.I.M.L.T. or F.I.M.L.T. or their equivalents. Considerable experience in histopathological techniques is essential.

The travelling expenses of a successful overseas applicant will be met on a pre-arranged basis.

Applications giving full details of training and experience should be submitted to the Director, South African Institute for Medical Research, P.O. Box 1038, Johannesburg, South Africa.

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG JOINT DENTAL RESEARCH UNIT OF THE COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH AND THE UNIVERSITY SENIOR TECHNICIAN

Applications are invited for appointment as Senior Technician on the staff of the joint C.S.I.R./University Dental Research Unit, which is housed in the University Oral and Dental Hospital, Milner Park, Johannesburg. Applicants should have had experience of histological methods as well as of general laboratory management. Skill in micro-photography and ability to handle animals will be an advantage. Duties are to be assumed on 1st November, 1957, or as soon as possible thereafter.

The salary attached to the post will be on the scale £750×50—£1,150 per annum (with an efficiency and long service barrier at the notch £950 per annum), plus a temporary cost of living allowance of £234 per annum in the case of a married man.

Membership of the Provident Fund is compulsory and involves a contribution of 7% of the salary, the Government and the University together contributing an equal amount. Membership of the Staff Medical Aid Fund is obligatory in the case of an officer who is found eligible for membership.

Applications, together with copies of testimonials and full particulars of qualifications and experience, must be lodged with the Registrar, University of the Witwatersrand, Milner Park, Johannesburg, not later than 25th September, 1957.

ABSTRACT

A Medical Technology Training Program Study. Houde, Ruth F. *Am. Journ. Med. Tech.* 23: 3, 184.

A critical review of the training of medical technologists at the University of Minnesota during some thirty-five years. Whilst much of this is applicable only to the American system there is much to be gleaned from it which could be of value when training programmes are reviewed here in South Africa. This is particularly true of the discussion on laboratory aides.

NOTICE

The Editor acknowledges receipt of a communication from Mr. L. P. Heitmann, which will receive attention in the next issue of the Journal.

NOTICE TO CONTRIBUTORS

All contributions are to be addressed to:—The Editor, The South African Journal of Medical Laboratory Technology, Room 213, Dept. of Pathology, Medical School, Umbilo Road, Durban.

Contributions may be written in English or Afrikaans, and should preferably be typed in double-spacing on foolscap sheets on one side of the paper only.

Figures should be drawn in Indian ink, and all figures and tables should be labelled as such (e.g. Figure 1, Table 1, etc.).

Authors should make adequate references to previous works on their subjects. These should be set out as follows:—Author's surname and initials of Christian names; the year of publication (in parentheses); the name of the journal, which should be abbreviated according to the World List of Scientific Periodicals (see below); the volume number (underlined); and the first page reference.

Example:—Moron, I. B. (1960). J. unsuccess. Med., **20**, 99. References to books should give the author's name and initials, the year of publication, title of book, name of publisher, and town in which published.

References should be arranged in alphabetical order of the authors' surnames. If more than one work by the same author is listed, these should appear in chronological order.

Technologists are reminded that regulations demand that all original articles of a technical or scientific nature must be approved by the heads of their departments before being submitted for publication.

Title abbreviations according to World List of Scientific periodicals

All nouns commence with capital letters, and adjectives small letters. Articles, conjunctions and prepositions are omitted.

Examples:—

J. Amer. med. Ass.	*S. Afr. J. clin. Sci.*
Lancet	*Stain Tech.*
Amer. J. clin. Path.	*J. Bact.*

REPRINTS AND PHOTOGRAPHS

If requested before publication, 24 reprints of original articles will be supplied free to contributors. As a temporary measure, contributors are asked to defray the costs of publishing diagrams and photographs accompanying articles.

KENNISGEWING AAN INSENDERS

Alle bydrae moet as gevolg geadresseer word: Die Editeur, Die Suid Afrikaanse Joernal van Mediese Technologie, Kamer 213, Dept. van Patologie, Mediese Skool, Umbiloweg, Durban, Natal.

Bydrae mag in Engels of Afrikaans geskryf word en moet verkieslik getik wees dubbel spasiering op folio-papier en net op een kant van die vel.

Figure moet in Indiese ink geteken word en alle figure en tabelle moet geëtikeer word as sulks (b.v. Figuur 1, Tabel 1, ens.).

Auteurs moet voldoende referensies gee tot vorige werke oor hulle onderwerpe. Die moet as volg uiteengesit word:—Auteur se familienaam en voorletters; die jaar van uitgawe (in hakies); die naam van die Joernaal, wat moet verkort volgens die Wêreld Lys van Wetenskaplike Tydskrifte (sien hieronder)r die volume nommer (onderstreep); en die eerste pagina referensie.

Voorbeeld:—Moron, I. B. (1960). J. unsuccess. Med., **20**, 99. Referensies tot boeke moet die auteur se naam en voorletters meld, die jaar van uitgawe, titel van boek, naam van uitgewer, en stad waar dit gepubliseer is.

Referensies moet in alfabetiese orde, volgens auteurs se familienaam gerangskik word. Indien meer dan een werk deur dieselfde auteur gemeld word, moet dit in tydsorde voorkom.

Tegnoloë word daaraan herinner dat regulasies vereis dat alle oorspronklike artikels van tegniese of wetenskaplike aard moet die goedkeuring dra van hulle departementale hoofde voor dit ingestuur word vir publikasie.

Titel verkortings volgens Wêreld Lys van Wetenskapliker Tydskifte

Alle selfstandige naamwoorde moet begin met hoofletters en byvoeglike naamwoorde met klein letters. Artikels, verbindings, en voorsetsels word uitgelaat.

Voorbeelde:—

J. Amer. med. Ass.	*S. Afr. J. clin. Sci.*
Lancet	*Stain Tech.*
Amer. J. clin. Path.	*J. Bact.*

HERDRUKKE EN FOTOGRAWE

Indien aanvraag ingedien word voor publisering, sal 24 herdrukke van oorspronklike artikels vry aan beydraers verskaf word. As in tydelike maatreel word bydraers gevra om die koste van publisering van fotos en tekeninge wat saam met artikels gaan self te betaal.

LA388S.A

It shows, in clear print, the full specification of the contents. You know exactly what you have got, you can see its field of use, and you have all the information handy for future reference.

All **M&B** Laboratory Chemicals and Reagents—there are now well over 550 of them—are produced to published standards of purity.

It all adds up to a convenient, reliable range of chemicals of wide application in general laboratory practice.

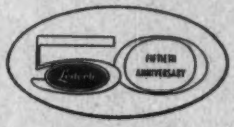
Further information will gladly be supplied on request.

MANUFACTURED BY
MAY & BAKER LTD

M&B
BRAND
LABORATORY
CHEMICALS
AND REAGENTS

DISTRIBUTORS:
MAYBAKER (S.A.) (PTY.) LTD · P.O. BOX 1130 · PORT ELIZABETH
TEL. 89011 (3 LINES)

When you want to control OEDEMA think first of . . .



Diamox*

Acetazolamide Lederle

A nonmercurial oral diuretic. Acts by inhibiting the enzyme carbonic anhydrase. Produces prompt, ample diuresis lasting from six to twelve hours. Morning dosage allows an uninterrupted night's sleep. Well-suited to long-term use. Nontoxic.

The most widely prescribed drug of its kind!

Indicated in cardiac oedema, epilepsy, acute glaucoma, pre-menstrual tension, oedema associated with toxaemia of pregnancy and oedema caused by certain types of electrolytic imbalance. Offered in scored tablets of 250 mg. for oral use.

PACKED IN BOTTLES OF 25 AND 100

* *Regd. Trade Mark*

LEDERLE LABORATORIES DIVISION
AMERICAN **CYANAMID** *COMPANY*
PEARL RIVER NEW YORK

Lederle

Sole South African Distributors:

ALEX LIPWORTH LIMITED

JOHANNESBURG - CAPE TOWN - DURBAN